CLINICAL STUDY REPORT

THE TEDIV STUDY

A Phase IIb, dual-centre, randomised, double-blind, comparator-controlled, parallel-group, pilot study of CaEDTA added to inhaled tobramycin vs tobramycin alone as adjunctive therapy to a course of standard treatment for cystic fibrosis patients admitted to hospital with a *Pseudomonas aeruginosa* pulmonary exacerbation

Investigational product	CaEDTA nebulised formulation
Indication	Cystic fibrosis
Protocol no.	TEDIV-001
Sponsor	Telethon Kids Institute
Study period	March 2014 – June 2016
Principal Investigator	Barry Clements

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1 Synopsis

Title	A Phase IIb, dual-centre, randomised, double-blind, comparator-controlled, parallel-group, pilot study of CaEDTA added to inhaled tobramycin vs tobramycin alone as adjunctive therapy to a course of standard treatment for Cystic Fibrosis patients admitted to hospital with a *Pseudomonas aeruginosa* pulmonary exacerbation
Brief title	A study to evaluate the efficacy of CaEDTA as an adjuvant to tobramycin therapy for *Pseudomonas* exacerbations in patients with cystic fibrosis
Clinical phase and study type	Phase 2 efficacy and safety
Objectives	Efficacy - Greater reduction in bacterial load - Greater clinical improvement Safety and tolerability - No adverse effects on respiratory symptoms or lung function - No evidence of systemic reaction or side effects
Endpoints	Efficacy - Change in colony forming units of *Pseudomonas aeruginosa* per gram sputum - Change in lung function (FEV1) - Change in CFQ-R questionnaire score Safety and tolerability - Post-dose lung function (FVEV1) - Adverse events - Clinical laboratory values (kidney and liver function, iron, calcium, magnesium)
Number of subjects	26 screened; 22 completed the study
Study population	Subjects with CF aged ≥6 years
Study drug	**Active substance:** CaEDTA in TRIS-buffered saline **Activity:** chelation of metal ions **Strength:** 75 mg in 4 ml **Route of administration:** nebulised, together with tobramycin (250 mg/dose) or with saline **Placebo:** Saline **Activity:** Placebo **Strength:** Normal saline (0.9%) **Route of administration:** nebulised, together with tobramycin (250 mg/dose) or on its own
Study duration	Total of 10 weeks - Inpatient treatment phase: 2 weeks - Outpatient treatment phase: 4 weeks - Safety phase: 4 weeks
Study design	Phase 2, randomized, placebo-controlled, double blind, parallel-group, dual centre study in subjects with CF who present with an exacerbation that requires a 2-week course of IV and nebulised Tobramycin treatment in hospital followed by a 4-week outpatient course of nebulised Tobramycin. The study is divided into three parts - Inpatient treatment phase (2 weeks): 4 doses of study drug (active or placebo) per day of which two were study drug alone and two in combination with tobramycin - Outpatient treatment phase (4 weeks): 2 doses of study drug per day in combination with tobramycin - Safety phase (4 weeks): no drug treatment

Summary and conclusions

Efficacy results

- Compliance was >90% throughout the study and was similar in both study groups.
- Treatment with nebulised CaEDTA in combination with tobramycin reduced bacterial counts in sputum faster than treatment with tobramycin alone. The mean reduction in colony counts over two weeks was $\log_{10}$ 2.9 in the CaEDTA group vs. $\log_{10}$ 0.7 in the placebo group.
- The mean improvement in FEV1 from 0 to 2 weeks was 16% in the CaEDTA group vs 5% in the placebo group. At the safety visit, four weeks after the end of treatment, the mean improvement was 7% in the CaEDTA group vs 2% in the placebo group.
- The CFQ-R questionnaire did not show a clear difference between the groups.

Safety and tolerability results

- Pre- and post-dose FEV1 showed +3% difference in the CaEDTA group and -1% in the placebo group. No individual patient showed any significant change associated with administration of either CaEDTA or placebo.
- Adverse events were very similar between both groups, and none were clearly linked to the study drug.
- No differences were observed in blood parameters between the two groups.
- PK data in three patients showed that only 2-5% of peak CaEDTA concentrations remained in sputum after two hours.

Conclusions

Overall, the CaEDTA nebulised solution was well tolerated, and no differences were observed in any safety endpoints between the active drug and placebo group. The study was not powered to show statistically significant differences for efficacy or safety. However, there was a greater mean reduction in sputum bacterial load in the CaEDTA group vs placebo, as well as a substantially greater mean improvement in FEV1 after two weeks. The results from this pilot study therefore support further exploration of the potential for combined use of inhaled CaEDTA and tobramycin in the treatment of pulmonary exacerbations in CF patients with chronic *Pseudomonas aeruginosa* infection.

2 List of abbreviations

AE	Adverse event
CaEDTA	Calcium ethylenediamine tetraacetate
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CFQ-R	Cystic Fibrosis Questionnaire-Respiratory domain
CFU	Colony forming Unit
EDTA	Ethylenediamine tetraacetate
FDA	US Food and Drug Administration
FEV1	Forced Expiratory Volume in one second
N/A	Not applicable
PMH	Princess Margaret Hospital
SAE	Serious adverse event
SCGH	Sir Charles Gairdner Hospital
Tris	Tris(hydroxymethyl)aminomethane
URTI	Upper respiratory tract infection

3 Ethics

Randomised placebo-controlled, double-blind, dual-centre study conducted at Princess Margaret Hospital and Sir Charles Gairdner Hospital, Perth, Australia. The study protocol was approved by Institutional Ethics Committee and Governance Review Boards at both sites. Informed consent was obtained from the parent and/or patient prior to commencing the study.

4 Introduction

4.1 Rationale and aims

Individuals with cystic fibrosis (CF) are highly susceptible to chronic lung infection by the opportunistic environmental bacterium *Pseudomonas aeruginosa*. The organism persists by forming biofilms within the relatively hypoxic mucous of the CF lung. Once established, these biofilms are virtually impossible to eradicate with existing therapies. For some time now it has been recognised that iron is essential for *P. aeruginosa* growth and biofilm formation.[1,2] CF lungs are known to contain up to 400 times more iron than normal lungs, which may contribute to the growth and persistence of invading bacteria.[3] In addition, *in vitro* studies have shown that removal of iron using metal ion chelators prevents biofilm formation and sensitises bacteria to enhance antimicrobial killing up to 1000 times.[4-6] This effect has been exploited for many years in the veterinary industry, which has used EDTA as an adjuvant to antibiotics.[7-9] However, EDTA has rarely been used to treat infections in humans. Only two previous studies have attempted to use it against *P. aeruginosa* lung infections, one of which successfully cleared *P. aeruginosa* pneumonia in four intubated patients in intensive care, where antibiotics alone had failed.[10]

Given the *in vitro* evidence and veterinary success, we hypothesised that addition of CaEDTA to nebulised tobramycin would result in improved clearance of *P. aeruginosa* from CF airways compared to treating with inhaled Tobramycin alone. There was sufficient safety evidence in animals and in humans where high-dose inhaled (Ca)EDTA had been used for heavy metal detoxification in patients over-exposed to heavy metals such as lead, to allow this clinical study to be performed. In this study, we evaluated the safety and efficacy of CaEDTA in treatment of pulmonary exacerbations in CF patients with chronic *P. aeruginosa* infection.

4.2 Target population

Patients with CF, ≥6 years old, chronically colonised with *P. aeruginosa*, presenting with an exacerbation that required treatment in hospital. There are several definitions for chronic *P. aeruginosa* colonisation. For the purpose of this study, chronic colonisation was defined as isolation of *P. aeruginosa* in more than 50% of airway samples over a 12-month period.[11]

4.3 Treatment and duration

The study period was divided into three phases: an inpatient treatment phase (2 weeks), an outpatient treatment phase (4 weeks), and a safety phase (4 weeks). During the inpatient phase, participants received study treatment (CaEDTA or placebo) four times daily – twice daily combined with their inhaled tobramycin, and twice daily on its own. This was done because mucociliary clearance reduces drug levels in the airway to less than 10% of peak concentrations within 2 hours of inhalation. Therefore, we felt we could at least increase the frequency of peak mucus CaEDTA concentrations each day while the patients were in hospital with plenty of time and support for the extra nebulisations. During the outpatient phase, participants continued to receive study drug (CaEDTA or placebo combined with tobramycin) twice daily.

4.4 Endpoints

The efficacy endpoints are change in colony forming units of *P. aeruginosa* per gram of sputum, change in lung function (FEV1) and change in CFQ-R questionnaire score. Safety endpoints include post-dose lung function, adverse events and clinical laboratory values.

5 Study objectives

To assess the efficacy, safety and tolerability of adding CaEDTA to inhaled tobramycin as adjunctive therapy to a standard course of antibiotics for the treatment of *P. aeruginosa* pulmonary exacerbation in patients with CF.

6 Investigational plan

6.1 Study design and plan

The TEDIV study was a dual-centre, randomised, double-blind, parallel-group, comparator study of inhaled Tobramycin with added CaEDTA vs inhaled Tobramycin with saline (placebo) for children and adults with cystic fibrosis (CF) admitted to hospital for treatment of a *P. aeruginosa* exacerbation.

All enrolled patients received the standard institutional treatment of pulmonary exacerbations with intravenous antibiotics and supportive therapy with some patients also receiving oral antibiotics during the 4-week outpatient phase. The choice of intravenous antibiotics and any additional antibacterial and/or antifungal agents (both intravenous and/or oral, and in and/or out of hospital) was made on clinical and microbiological grounds by the treating physician and was not influenced by the trial.

In addition to standard care, patients were randomised to receive inhaled tobramycin along with either CaEDTA (active) or saline (placebo) according to a three-phase study design (Figure 1):

1) An inpatient phase, during which both intravenous and nebulised antibiotics were given
2) An outpatient phase, during which patients received nebulised antibiotics
3) A follow-up phase to monitor safety and ongoing efficacy.

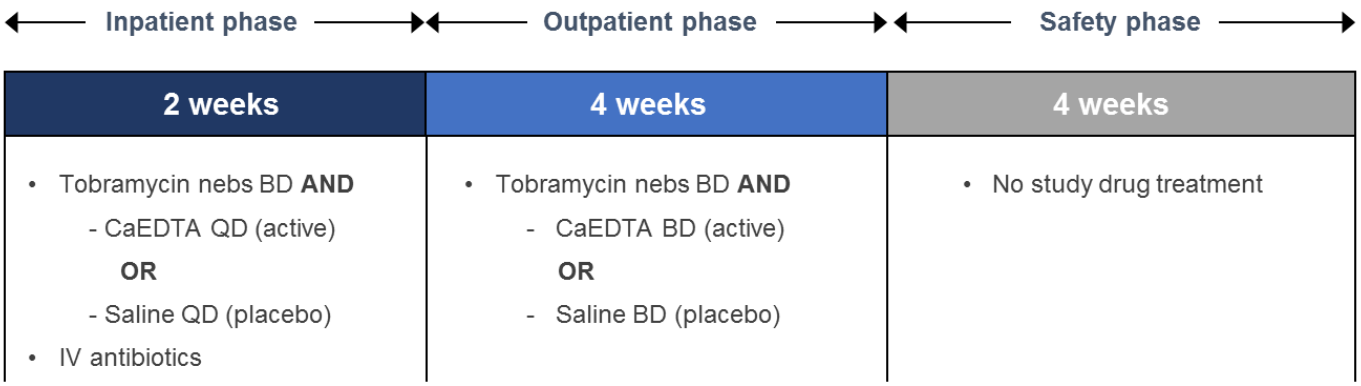

Figure 1. Study design.

During the 2-week inpatient phase while patients were treated with IV antibiotics, participants received study treatment (CaEDTA or placebo) four times daily – twice daily combined with their inhaled antibiotic, and twice daily on its own in between the inhaled antibiotic doses. The rationale behind the two extra daily doses of CaEDTA (or placebo) during the intravenous phase was to increase the period of peak CaEDTA airway activity while the antibiotic was presented to the airway mucosa in a continuous fashion via the bloodstream over the 24-hour period of each day, in addition to the twice daily nebulised Tobramycin.

Following discharge from hospital - during the 4-week outpatient phase - participants continued to receive study drug (inhaled Tobramycin with added CaEDTA or placebo) twice daily. Baseline assessments and measurements, as well as safety and tolerability, was evaluated on day 1 and safety and efficacy assessments were carried out weekly on day 8 and day 15 until discharge. Subsequent assessments in the outpatient phase were carried out by telephone call visits on day 29 and a clinic visit on day 43. Telephone visits were used to follow general progress and focused on tolerability, side effects and adherence, while clinical visits monitored safety and efficacy parameters.

Four weeks after the end of treatment, patients attended a visit on day 71 for final safety and efficacy assessment. An overview of clinical study visits and assessments is shown in Table 1.

Table 1. Overview of study visits and evaluations

	Inpatient phase				**Outpatient phase**		
Visit	**Screening**	**Visit 1**	**Visit 2**	**Visit 3** Discharge	**Visit 4** Phone call	**Visit 5** End of treatment	**Follow-up** Safety visit
Days ± visit window	**Day -3 to +1**	**Day 1**	**Day 8 ± 3**	**Day 15 ± 3**	**Day 29 ± 3**	**Day 43 ± 3**	**Day 71 ± 7**
Informed consent	x						
Eligibility criteria	x						
Demographics	x						
Medical history	x	x					
Respiratory symptoms check	x	x	x	x	x	x	x
Height and weight	x		x	x		x	x
Vital signs	x		x	x		x	x
Physical exam	x		x	x		x	x
Spirometry	x	x x x[1]	x x[2]	x x[2]		x	x
Sputum collection	x			x		x	x
Blood collection	x			x		x	x
Concomitant medications	x	x	x	x	x	x	x
CF Questionnaire				x		x	x
Dispense medication		x		x			
Adverse events		x	x	x	x	x	x
First dose of study medication		x					
Patient observation (0.5, 1 and 2 hours post-dose)		x					

[1]At visit 1, spirometry will be performed pre-dose and at ½, 1 and 2 hours post-dose.
[2]At visits 2 and 3, spirometry will be performed pre-dose and post-dose.

6.2 Selection of study population

Eligible patients admitted to hospital for treatment of pulmonary exacerbation with intravenous antibiotics were approached with the treating physician's approval to participate in the trial.

6.2.1 Inclusion Criteria

- Male or female ≥6 years of age with a documented diagnosis of CF (positive sweat chloride test, genotype with two identifiable CF mutations) accompanied by one or more clinical features consistent with the CF phenotype
- Current pulmonary exacerbation requiring in-patient antibiotic therapy
- Able to perform acceptable spirometric manoeuvres
- FEV1 > 25% of predicted values
- Positive sputum or bronchoalveolar lavage culture for *P. aeruginosa* in the past 12 months
- Ability to give informed consent or have legally acceptable representative who can give informed consent in accordance with ICH/GCP
- Females of child-bearing potential must agree to use an acceptable method of contraception for the duration of the trial.

6.2.2 Exclusion Criteria

- Known hypersensitivity to the investigational product or its components or known relevant medication allergy
- Participation in another study with an investigational drug within 2 months of the planned first dose of investigational product.
- Known relevant substance abuse.
- Female patients who are pregnant or lactating
- Clinically significant disease or other medical condition other than CF or CF related conditions that would, in the opinion or the Investigator, compromise the safety of the patient or quality of the data.

The presence of additional bacterial or fungal organisms on sputum culture and/or the prescription of additional antibiotics (oral, intravenous, antipseudomonal, or non-antipseudomonal) at any stage through the trial did not affect inclusion.

6.3 Treatments

6.3.1 Investigational product and comparator

The active component of the study drug is Calcium disodium ethylenediamine tetraacetate ($CaNa_2EDTA$, abbreviated CaEDTA). Since nebulised tobramycin is part of the standard treatment of *P. aeruginosa* exacerbations, CaEDTA was co-administered with tobramycin in a single nebulised solution.

The formulation was prepared by dissolving CaEDTA (the active drug) and tris in 0.9% saline and adjusting the pH to 7.1. The study drug was delivered to patients in two ampoules – one containing tobramycin (250 mg in 2.5 ml), the other containing either CaEDTA (75 mg in 1.5 ml; 50 mM) or tris buffered saline alone (1.5 ml, placebo). The two ampoules were combined into one 4 ml nebulising solution, which was delivered using a Pari-LC plus nebuliser until completion.

The active drug was co-administered with tobramycin twice a day throughout the study period, as well as twice a day on its own during the inpatient phase. In the latter case, 1.5 ml of the study drug or placebo was added to 2.5 ml 0.9% saline to a final volume of 4 ml.

The investigational product and comparator were manufactured and stored at the PMH Pharmacy Department.

6.3.2 Randomisation and blinding

Prior to commencement of the study, the pharmacist prepared a permuted-block randomisation schedule. The statistician held the full randomisation code, so that all investigators, study staff and participants remained blinded. Access to a patient's allocated treatment was available in a sealed envelope, kept in the pharmacy with the investigational product should there be a need for un-blinding.

6.3.3 Prior and concomitant therapy

In addition to the inhaled study drug, patients continued with their standard CF care and medications (including any additional antibiotics at the discretion of the treating physician, as well as other forms of inhaled therapy such as Pulmozyme and hypertonic saline if necessary). All concomitant medications were recorded throughout the study.

6.3.4 Treatment compliance

Compliance was monitored at each visit with the use of charts, simple questioning, diary cards and medication counts.

6.4 Efficacy and safety variables

The primary efficacy variable was a reduction in the number of *P. aeruginosa* CFU per gram sputum. Sputum samples were collected, processed and frozen, then spread on McConkey agar, which is selective for *P. aeruginosa*, and enumerated.

FEV1 was measured by spirometry at the indicated visits. Post-dose FEV1 was monitored as a safety outcome, and further measurements were made throughout the study as an exploratory efficacy outcome. Clinical parameters were monitored using the CFQ-R questionnaire, which was scored and used as an exploratory outcome measure.

Blood parameters were monitored throughout the study for safety, particularly renal function (creatinine), liver function (ALT and GGT), and minerals (calcium, magnesium and iron-related parameters). Adverse events were recorded and monitored throughout.

Finally, pharmacokinetic data was collected in three patients by measuring EDTA levels (by LC-MS) in sputum 5 minutes and 2 hours post-dose.

6.5 Statistical methods

Data was analysed using summary statistics and results were presented as means (standard deviation) or median (range).

6.6 Changes in the conduct of the study

Due to slow recruitment of paediatric patients, a second study site (SCGH) was added to include adult patients.

Some of the exploratory endpoints were not analysed due to insufficient sputum samples from each patient.

7 Study participants

7.1 Disposition of patients

Of 26 randomised patients, 24 patients received the study drug and 22 completed the study procedures through to 10 weeks (Figure 2 and Table 2). One patient in each group withdrew consent following randomisation and never received the study drug, and one in each group was lost to follow-up after 2 and 7 days of treatment respectively.

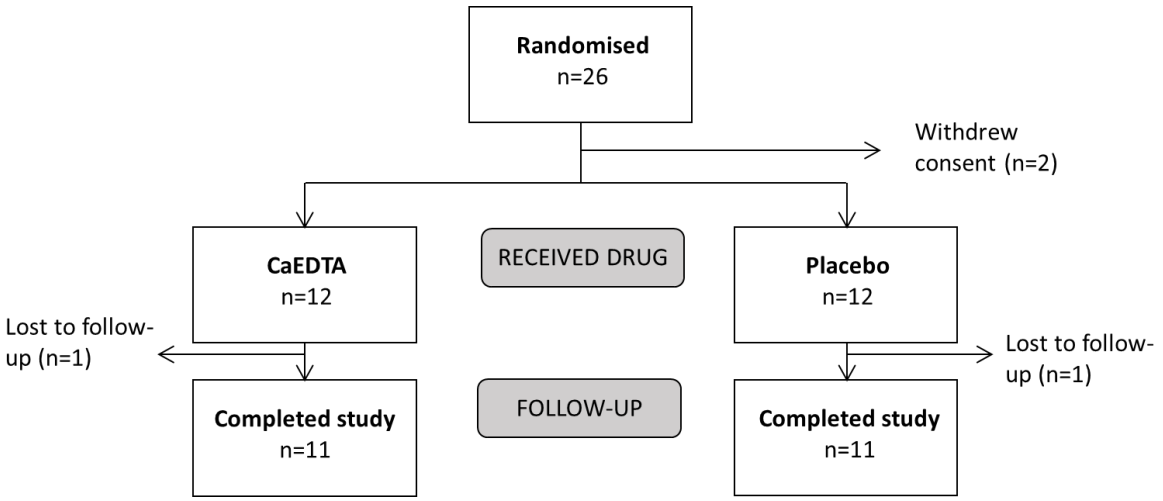

Figure 2. Patient flow diagram

Table 2. Study overview of individual patients.

Patient identifier	Study group	Received study drug	Completed study	Comments
TEDIV 001-01	CaEDTA	Yes	Yes	Excluded from efficacy analysis after 2 weeks due to non-compliance while an outpatient.
TEDIV 001-02	Placebo	Yes	Yes	
TEDIV 001-03	Placebo	Yes	Yes	
TEDIV 001-04	CaEDTA	Yes	Yes	
TEDIV 001-05	Placebo	Yes	Yes	
TEDIV 001-06	CaEDTA	Yes	Yes	Negative cultures throughout - excluded from efficacy.
TEDIV 001-07	CaEDTA	No	No	Withdrew consent after screening on day 1.
TEDIV 001-08	Placebo	Yes	No	Lost to follow-up on day 8 after 7 days of treatment. Excluded from all analyses apart from FEV1 safety data.
TEDIV 001-09	CaEDTA	Yes	No	Lost to follow-up on day 2 for personal reasons. Excluded from all analyses apart from FEV1 safety data. Patient re-enrolled later (identifier 001-24).
TEDIV 001-10	Placebo	Yes	Yes	
TEDIV 001-11	Placebo	Yes	Yes	
TEDIV 001-12	CaEDTA	Yes	Yes	
TEDIV 001-13	CaEDTA	Yes	Yes	
TEDIV 001-14	Placebo	No	No	Withdrew consent on day 1.

TEDIV 001-15	Placebo	Yes	Yes	
TEDIV 001-16	CaEDTA	Yes	Yes	
TEDIV 001-17	Placebo	Yes	Yes	
TEDIV 001-18	CaEDTA	Yes	Yes	
TEDIV 001-19	Placebo	Yes	Yes	
TEDIV 001-20	CaEDTA	Yes	Yes	
TEDIV 001-21	CaEDTA	Yes	Yes	
TEDIV 001-22	Placebo	Yes	Yes	
TEDIV 001-23	Placebo	Yes	Yes	Negative cultures throughout - excluded from efficacy.
TEDIV 001-24	CaEDTA	Yes	Yes	
TEDIV 001-25	Placebo	Yes	Yes	No sputum produced at any visit - excluded from efficacy.
TEDIV 001-26	CaEDTA	Yes	Yes	

7.2 Protocol deviations

Some protocol deviations were noted throughout the study. Three patients were withdrawn from the study, two because consent was withdrawn and one because the patient discharged herself and the study team was unable to reach her again. In one case, the study drug was stopped temporarily for 12 hours due to an adverse event.

The remaining deviations were related to the timing of scheduled events. One patient started the study drug 17 hours outside the study window, and two patients had visit 6 conducted outside the study window. Further detail can be seen in Appendix 12.4.1.

8 Efficacy evaluation

8.1 Data sets analysed

Data for the efficacy analyses can be seen in Appendix 12.4. Unless otherwise stated, all 22 patients who completed the study procedures through to 10 weeks were included in the analyses.

Patient 1 was excluded from the efficacy and FEV1 analysis after the two-week hospital inpatient period due to subsequent non-compliance at home. The data from the first two weeks was included for both efficacy and safety.

For the microbiology, 3 of the patients who completed the study were excluded from the analysis. Patient 25 (placebo group) produced no sputum sample for the duration of the study. Patients 6 (CaEDTA group) and 23 (placebo) only produced samples that were culture negative for *P. aeruginosa*. Furthermore, patient 1 was excluded after 2 weeks due to non-compliance.

8.2 Demographic data

Key demographic data are outlined in Table 3. The study groups were well matched in baseline parameters, including weight, sex, pancreatic status, and hospital admissions in the previous 12 months. At screening, the CaEDTA group was slightly older, had a lower FEV1 and a higher bacterial load per gram sputum.

Table 3. Baseline demographic data and clinical characteristics.

	Placebo **n=12**	**CaEDTA** **n=12**	**Total** **n=24**
Age (years); mean (SD)	18.9 (9.6)	22.3 (12.7)	20.6 (11.1)
Age group; n (%)			
6-13 years	4 (33.3%)	3 (25%)	7 (29.2%)
14-17 years	4 (33.3%)	4 (33.3%)	8 (33.3%)
>18 years	4 (33.3%)	5 (41.6%)	9 (37.5%)
Male gender; n (%)	6 (50%)	8 (67%)	14 (58%)
Pancreatic insufficient; n (%)	10 (83%)	12 (100%)	22 (92%)
Baseline weight (kg); median (range)	49 (20-73)	63 (23-77)	52 (20-77)
Baseline values			
FEV_1 (%predicted); mean (SD)	72 (23)	53 (27)	63 (27)
Log_{10} *P.aeuginosa* (CFU/g sputum); mean (SD)	3.8 (2.5)	5.0 (2.0)	4.4 (2.3)
CFQ-R score; mean (SD)	19 (3.0)	18 (2.7)	19 (2.8)
Hospital admissions in previous 12 months; mean (SD)	1.0 (0.95)	1.3 (1.2)	1.1 (1.1)

8.3 Compliance to treatment

The analysis of compliance was separated into the two study periods, the inpatient phase and the outpatient phase. As outlined in Table 4, the inpatient phase lasted two weeks with a QID treatment regime, and the outpatient phase lasted four weeks with a BID treatment regime, corresponding to 56 doses for each phase.

Of the available data, compliance was high with the average above 90% for the duration of the study (Table 4). Furthermore, there was no difference between the closely monitored inpatient phase and the outpatient phase. Finally, no difference was observed in compliance between the treatment and placebo groups, supporting excellent tolerability of the study drug and that it was as acceptable to patients as placebo.

Table 4. Overview of compliance.

	Inpatient phase			**Outpatient phase**		
Cohort	**No. doses; mean (SD)**	**Compliance (%); mean (SD)**	**No. patients in analysis**	**No. doses; mean (SD)**	**Compliance (%); mean (SD)**	**No. patients**
Placebo	53 (3.2)	94 (5.8)	9	51 (4.5)	92 (8.1)	10
CaEDTA	51 (4.7)	91 (8.5)	11	53 (2.7)	94 (4.8)	6

8.4 Efficacy evaluation

8.4.1 Reduction in bacterial load

As outlined in Table 1, the bacterial load of *P. aeruginosa* in sputum was measured at baseline, at two weeks (before discharge), six weeks (end of treatment), and at 10 weeks (safety visit). The results show that the mean reduction in colony forming units (CFU) after two weeks is $\log_{10}$ 2.9 in the CaEDTA group vs. $\log_{10}$ 0.7 in the placebo group. The reduction in the CFU count in the CaEDTA group was maintained throughout the study in the CaEDTA group, while the mean reduction in CFU counts in the placebo group reached $\log_{10}$ 1.9 at 10 weeks.

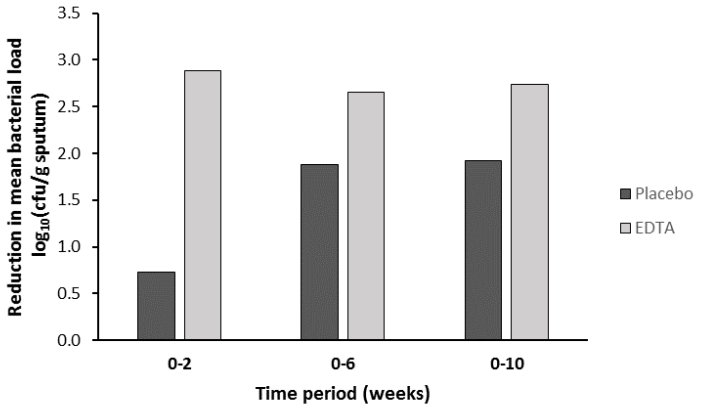

Figure 3. Reduction in *P. aeruginosa* colony forming units from baseline.

The data is somewhat limited by a reduced number of samples at the later visits. This can likely be attributed to clinical improvement, since patients are less likely to produce sputum after recovery from the exacerbation event. Despite this limitation, the trends are clear – especially during the first two weeks. Of the patients who had sputum samples for both baseline and two weeks, and at least one positive culture for *P. aeruginosa*, only 1 of 7 in the CaEDTA group (14%) had an increase in bacterial load over the first two weeks, compared to 4 of 8 (50%) in the placebo group. The full data is shown in Appendix 12.4.4.

8.4.2 Clinical improvement – FEV1

Lung function, as measured by FEV1 (% predicted), was monitored throughout the study. As expected, the mean FEV1 increased over time in both groups, although the mean increase in FEV1 in the CaEDTA cohort was 16 % points predicted in the first two weeks vs 5% in the placebo group (Figure 4).

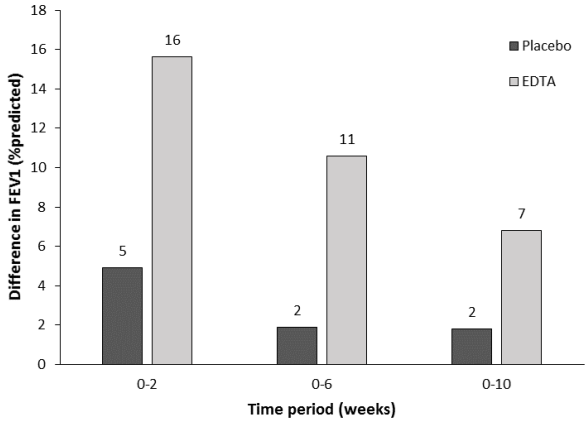

Figure 4. Change in FEV1 from baseline.

A multivariate linear regression model shows the same trend, albeit of a slightly more modest magnitude. According to this model, the mean FEV1 improvement in the CaEDTA group was 6.4% higher than in the placebo group in the same period. Full data is available in Appendix 12.4.5.

8.4.3 CFQ-R questionnaire

Clinical improvement was also assessed using the CFQ-R questionnaire (example in Appendix 12.3, full data in Appendix 12.4.6). Responses were scored 0-4 with 0 representing an improvement and 4 representing a worsening in clinical situation. A low overall score therefore represents a good clinical outcome.

Table 5 gives an overview of the CFQ-R over time. Both groups improved during the study, but no striking differences were seen between the groups.

Table 5. CFQ-R scores.

	Time (weeks)			
Cohort	**0**	**2**	**6**	**10**
Placebo, mean (SD)	19 (3)	12 (4)	14 (6)	14 (4)
CaEDTA, mean (SD)	18 (3)	13 (5)	17 (4)	15 (4)

8.5 Efficacy conclusions

The two study groups were generally well matched demographically and in disease severity. The main differences to note were that the CaEDTA group had a higher mean bacterial load and a lower mean lung function than the placebo group. Compliance was high in both groups throughout the study period, suggesting that the results are valid and that the active drug was tolerated as well as placebo. The efficacy data showed that the CaEDTA group had a higher reduction in mean bacterial load after two weeks than the placebo group (log_{10} 2.9 in the CaEDTA group vs. log_{10} 0.7 in the placebo group) along with a larger improvement in FEV1 in the CaEDTA group (16% mean improvement in FEV1 vs 5% in the placebo group). Although the study was not powered to show statistically significant differences, these results strongly suggest that the combination of CaEDTA and tobramycin benefitted patients more in recovery after an exacerbation than tobramycin alone.

9 Safety evaluation

9.1 Extent of exposure

Upon completion of the study, 11 patients had been exposed to the study drug at 4×75 mg/day for two weeks and 2×75 mg/day for four weeks. During the first two weeks (inpatient phase), half of the doses were CaEDTA alone, while all the remaining doses were in combination with tobramycin (250mg mg/dose). A 12th patient received the drug for one day before withdrawing consent. The concentration of active drug in the 4 ml nebulised solution was 50 mM CaEDTA.

Similarly, 11 patients were exposed to placebo (saline) four times a day for two weeks and then twice a day for four weeks. During the first two weeks, half of the doses were saline alone, while the remaining doses were in combination with tobramycin. A 12th patient received placebo for 7 days before withdrawing from the study.

9.2 Data sets analysed

Unless otherwise stated, all patients who received the study drug or placebo were included in the safety analyses. Complete data for the safety analyses can be seen in Appendix 12.4. For the FEV1 safety analysis, post-dose measurements were taken at 30, 60 and 120 min. Of the 24 patients who received the study drug, all were included in the FEV1 safety analysis apart from Patients 15, 18 and 24 who had no post-dose data (at least one due to technical problems with the spirometer).

If not otherwise stated, all 22 patients who completed the study were included in the blood safety analyses to the extent that data was available. Patient 20 was excluded from iron-related data analyses due to an iron infusion during the inpatient phase.

9.3 FEV1 prior and post administration

The first safety concern of inhaled drugs is the immediate effect on lung function as a result of deposition of the drug in the airways. Table 6 shows the trend in FEV1 in the first two hours following administration. The overall changes were minor in both groups.

Table 6. Reduction in FEV1 (% predicted) following drug administration.

	Time following administration		
Cohort	**30 min**	**1 hour**	**2 hours**
Placebo, mean (SD)	4.1 (4.8)	3.1 (2.9)	0.78 (3.8)
EDTA, mean (SD)	0.67 (3.6)	2.1 (3.0)	-0.38 (4.9)

9.4 Adverse events

Adverse events are outlined in Table 7 below. None of these were specifically attributed to the study drug, and most of them were mild. The most common complaint was headache, which was reported by four patients in each group. One patient in the CaEDTA group (patient 12) interrupted the drug temporarily due to hemoptysis, and one patient in the placebo group (patient 10) discontinued the drug shortly before the end of treatment due to viral illness. Both patients completed the study. Haemoptysis occurred in three patients, one in the CaEDTA group and two in the placebo group. Importantly, there was no difference in nature or severity between the study and placebo groups.

Table 7. Safety and adverse event profile.

Adverse event category	Placebo	CaEDTA	Total
Adverse event leading to study drug interruption	0	1	1
Adverse event leading to permanent discontinuation of study drug	1	0	1
Haemoptysis	2	1	3
Epistaxis	1	0	1
Increase in cough	3	0	3
Sore throat	3	1	4
Nausea/vomiting	2[†]	1	3
Abdomen pain	2	2	4
Rashes	3	0	3
Headache	4	4	8
Deranged LFT's	1	1	2
Admission to Hospital	2[‡]	0	2

[†]2 episodes in the same patient

[‡]Admissions related to viral illness, one of them with Respiratory Syncytial Virus infection

9.5 Clinical laboratory evaluation

The changes in renal function (monitored by serum creatinine) and liver function (monitored by alanine aminotransferase level (ALTY) and Gamma-glutamyl transferase (GGT) over the 10-week study period were generally low and insignificant, inferring that the CaEDTA did not have any adverse renal or hepatic side effects (Table 8).

Table 8. Mean changes in blood parameters compared to baseline.

	Placebo			CaEDTA		
Time period (weeks)	**0-2**	**0-6**	**0-10**	**0-2**	**0-6**	**0-10**
Creatinine	1.45	3.90	2.10	3.00	-2.25	0.43
Alanine aminotransferase	15.60	16.18	-2.11	8.00	1.11	-1.29
Gamma-glutamyl transferase	8.20	17.64	-0.29	4.33	20.78	1.43
Ferritin	-3.30	-16.50	-2.22	30.29	-2.11	-9.00
Iron	8.30	2.75	3.67	7.00	3.00	3.67
Transferrin	3.20	4.38	5.78	2.43	2.38	2.33
Transferrin saturation	12.40	3.38	5.33	9.86	4.00	5.00
Haemoglobin	0.10	5.36	2.10	-1.78	2.67	5.33
Blood urea nitrogen	3.26	0.63	1.00	1.80	0.30	0.80
Calcium	-0.01	0.03	-0.01	0.07	0.04	0.01
Magnesium	0.02	0.01	0.03	0.02	0.00	-0.03

One concern might be that CaEDTA not only removes excess metal ions in the airways, but may also reduce the levels of iron, calcium and magnesium in the systemic circulation. However, this was not observed as a result of inhalation of CaEDTA treatment; Calcium, magnesium, iron, transferrin and transferrin saturation levels were either unchanged or slightly increased in both groups compared to baseline. Ferritin levels were generally low, although mildly increased in the CaEDTA group after two weeks. The fact that ferritin does not follow the trends in other iron-related parameters is likely linked to its role in inflammation.

9.6 Pharmacokinetic data

Three patients provided sputum samples for pharmacokinetic evaluation. Samples were taken immediately before, as well as five minutes and two hours following a dose. EDTA was then measured in the sputum by LC-MS (Figure 5). The peak sputum concentration was in the millimolar range, but clearance was rapid and only 2-5% remained after two hours.

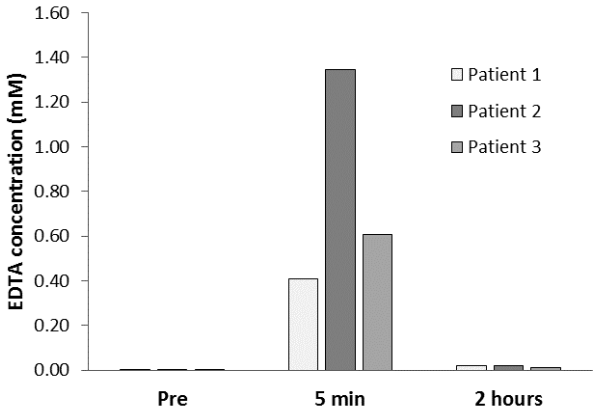

Figure 5. EDTA concentration in sputum before and after a dose.

9.7 Safety conclusions

Overall, inhalation of CaEDTA in combination with tobramycin did not raise any safety concerns. The mean reduction in FEV1 following administration for the treatment group was minor and even lower than for the placebo group. Although adverse events were noted throughout the study period, they were mostly mild and none were attributed to the study drug. Importantly, there was no difference in nature or severity of adverse events between the study and placebo groups. Blood tests showed no differences in renal or hepatic function, and no systemic depletion of iron, calcium or magnesium was observed. Finally, pharmacokinetic tests showed that peak sputum concentrations of CaEDTA reached the millimolar range after 5 minutes, but that clearance was rapid and only 2-5% remained after two hours.

10 Discussion and overall conclusions

The present study demonstrates synergistic effects of combined use of nebulised CaEDTA and tobramycin, in the treatment of pulmonary exacerbations in CF patients with chronic *P. aeruginosa* infection. Nebulisation of CaEDTA in combination with tobramycin resulted in greater reduction in the sputum load of *P. aeruginosa* compared to treating with tobramycin alone. This effect was expected and in agreement with published literature.[6,10,12] What was not expected, however, was the effect on FEV1, which showed a mean improvement of 16% in the CaEDTA group vs. 5% in the placebo group. In comparison, it is worth noting that the recently introduced CFTR modulator Orkambi shows FEV1 improvements of up to 4%. Findings from the study did not show any increased risk of adverse events in patients treated with nebulised CaEDTA compared to those treated with placebo, and no differences were observed in any of the safety endpoints.

The substantial improvement in lung function may suggest that CaEDTA has other positive effects in the lungs, beyond merely reducing bacterial counts in the sputum. One possible explanation could be that excess iron catalyses the generation of hydroxyl radicals, which are powerful triggers of inflammation. Removal of this iron may reduce oxidative stress and thereby lung inflammation. Another explanation could be that EDTA is a well-known inhibitor of matrix metalloproteinases (MMPs), the zinc-dependent activity of which is known to trigger host inflammatory responses and immunological responses.[13] Further work to investigate these potential alternative mechanisms of action is planned.

There are several limitations to the study that prevent definitive conclusions. The number of patients was too low to provide sufficient power for statistically significant efficacy endpoints. Other aspects that warrant further research are the effects on other microorganisms, dose optimisation, and the long-term efficacy and safety of inhaled CaEDTA.

Conclusion: Despite the limitations, the results from this study provide strong support for the potential of inhaled CaEDTA and tobramycin in the treatment of pulmonary exacerbations in CF patients with chronic *Pseudomonas aeruginosa* infection.

11 References

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12 Appendices

12.1 Parent information and consent form

Government of **Western Australia**
Department of **Health**
Child and Adolescent Health Service

Research Study

Adult Information Sheet

Study Title	A Phase IIb, Single-Centre, Randomised, Double-Blind, Comparator-Controlled, Parallel-Group, Pilot Study of Ca-EDTA added to Inhaled Tobramycin vs Tobramycin Alone as Adjunctive therapy to a Course of Standard Treatment for Cystic Fibrosis Children Admitted to Hospital with a *Pseudomonas aeruginosa* Pulmonary Exacerbation.
Study ID	TEDIV-001
Protocol Version	Protocol version 2.0 dated 16 August 2013
Principal Investigator	Dr Barry Clements
Institution	Princess Margaret Hospital for Children
Phone Number	9340 8830 or 9340 8222 (24-hr emergency)

The following information applies to the adult participant or to the child. If the participant is a child, the use of "you" refers to "your child." Children and adolescents will be given a separate information sheet to read.

We are asking if you would like to take part in this research study because you have cystic fibrosis (CF) and are growing a bug called *Pseudomonas aeruginosa* (*PsA*) in your lungs. This study is testing whether adding a small amount of a chemical called calcium edetate (Ca-EDTA) to your usual inhaled medicine will make the medicine work better.

Before you decide if you are willing to take part in this study, we want you to understand why it is being done, what you will have to do and how your information will be used. Please take time to read the information carefully and, if you wish, discuss it with friends, family and your doctor. One or more of our team members will go through this information sheet with you and answer any questions you have. Please ask questions about anything that you do not understand or want to know more about.

You do not have to do this study if you don't want to. Even if you decide to take part, you can still change your mind and stop doing the study. You do not have to give us a reason for stopping. Whatever you decide, it will not affect your routine treatment or future health care.

If you decide you want to take part in this study, you will be asked to sign the consent section of this form. By signing it you are telling us that you:

- understand what you have read
- consent to take part in this study
- consent to have the tests and treatments that are described in this information sheet
- consent for us to use your personal and health information as described in this information sheet

You will be given a copy of this information and consent form to keep.

Why are we doing the study?

Patients with CF often have lung infections which keep happening or get worse over time. These chronic lung infections may lead to worsening lung function. Lung infections are often caused by bacteria (germs). We treat this type of lung infection with antibiotics (a type of medicine that fights against bacteria). Antibiotics, such as tobramycin, either remove the bacteria, or stop or slow down their growth. This improves your cough and will help you to breathe. *Pseudomonas aeruginosa (PsA)* is a common bacteria that can cause lung infections in CF patients. Once it has become established in the airway, antibiotics alone will not be able to remove it. This is because the *PsA* protects itself by forming a slimy protective coat called biofilm. This biofilm stops the antibiotic from reaching and killing the *PsA*.

Calcium Edetate (Ca-EDTA) is a chemical that has been shown in the laboratory and in animal and human studies (but not in CF) to destroy the biofilm and to stop its production. If this happens, bacteria such as *PsA* are more likely to be killed by antibiotics. In this study, we want to see if adding Ca-EDTA to your inhaled tobramycin will remove the protective layer surrounding the bacteria in your lungs, and make it easier to kill.

Nebulised tobramycin by itself is approved to treat *PsA* lung infections in CF patients but adding Ca-EDTA to the tobramycin is an experimental treatment. This means that it has not been approved to treat CF lung infections, either in Australia or in other parts of the world. It must therefore be tested to see if it is safe and effective.

This study is designed to get information about how well the study treatment (tobramycin plus Ca-EDTA) works and how safe and well tolerated it is when given to CF patients to treat bacterial lung infections caused by *PsA.* If you participate in this study, no other aspect of your CF treatment will be altered. This includes your standard CF treatment and any other antibiotic or inhaled treatment your treating doctor may wish to prescribe.

How is the study designed?

To find out which is the best way of treating *PsA* lung infections we need to compare the study treatment (inhaled tobramycin with Ca-EDTA) to the normal treatment (inhaled tobramycin without Ca-EDTA). This type of study is called a comparator-controlled study.

There are two treatment groups. One group will receive the study treatment (inhaled tobramycin with Ca-EDTA) and the other group will receive the normal treatment (inhaled tobramycin without Ca-EDTA). Neither you nor the research staff will know which treatment group you are in. This is called double-blinding. The study doctor will be able to find out which treatment group you are in should it become necessary for medical reasons.

Patients are assigned to their treatment group by a computer. You have a 50:50 chance of being in either group. This is called randomisation.

Approximately 32 patients from Princess Margaret Hospital are expected to take part in this study. At the end of the study, if the 16 patients who received Ca-EDTA with their Tobramycin did better than the 16 who did not receive Ca-EDTA with their Tobramycin, then we know that treatment with Ca-EDTA works.

Study Drug

Ca-EDTA is a salt which can be dissolved in saline and injected into the veins or breathed into the lungs (inhaled). On this study the Ca-EDTA will be inhaled with your tobramycin. The Ca-EDTA will be added to the saline you add to the tobramycin.

Who is carrying out the study?

This study is being done at Princess Margaret Hospital under the direction of Dr Barry Clements and with the support and approval of all the consultants in the Respiratory Department. No member of the research team will be awarded for carrying out this study, other than their ordinary wages.

The funding for this study is coming from Dr Clements' research fund. He may also apply for scientific research grants in order to fund this study.

Do I have to take part?

You do not have to take part in this study if you don't want to. Before you decide if you want to take part, the study doctor or a member of his team will talk to you about all the options

available to you. You can feel free to ask any questions you like at this stage or at any stage throughout the study. If you decide not to take part, you will continue to receive the standard clinic treatment which you have always had.

What will I have to do if I decide to take part?

The first visit is a screening visit where we will see if you are eligible to be in the study. At this time (and at any time throughout the study) you can ask the study team any questions you may have. We will seek permission from your regular consultant doctor before asking you to participate in the trial.

The study runs for 71 days (10 weeks) with treatment for 6 weeks and one follow-up visit 4 weeks later to check how everything went and that everything is alright. The first two weeks of the study will take place while you are in hospital. Members of the study team will see you three or four times (approximately once a week) while you are on the ward. Once you are discharged from hospital we will ask you to continue taking the study medication for four weeks at home. There is one telephone call during this time and then one visit to PMH at the end of the four weeks when you will stop taking the treatment.. Finally, four weeks after you have stopped taking the trial medication, we will ask you to come in to PMH for a final follow-up visit.

Below is a table showing you what procedures will be done at each study visit.

Schedule of assessments – Outline of the visits and procedures for the TEDIV-001 study

	Hospital				At Home		
Visit	**Screening**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4 (TC)**	**Visit 5 (EoT)**	**Follow-up**
Days ± visit window	**Day 1 - 3**	**Day 1**	**Day 8 ± 3**	**Day 15 ± 3**	**Day 29 ± 3**	**Day 43 ± 3**	**Day 71 ± 7**
Informed consent	**X**						
Eligibility criteria	**X**						
Demographics	**X**						
Medical history	**X**	**x**					
Respiratory symptoms check	**X**	**x**	**x**	**x**	**x**	**x**	**x**
Height and weight	**X**		**x**	**x**		**x**	**x**
Vital signs	**X**		**x**	**x**		**x**	**x**
Physical exam	**X**		**x**	**x**		**x**	**x**
Spirometry	**X**	**x x x**[1]	**x x**	**x x**		**x**	**x**
Sputum collection	**X**						
Blood collection	**X**			**x**		**x**	**x**
Concomitant medications	**X**	**x**	**x**	**x**	**x**	**x**	**x**
CF Questionnaire				**x**		**x**	**x**
Dispense medication		**x**		**x**			
Adverse events		**x**	**x**	**x**	**x**	**x**	**x**
First dose of study medication		**x**					
Patient observation (1/2, 1 and 2 hours post-dose)		**x**					

TC = Telephone Call; EoT = End of Treatment

[1] At visit 1, spirometry will be performed pre-dose and at ½, 1 and 2 hours post-dose

[2] At visits 2 and 3, spirometry will be performed pre-dose and post-dose

Informed Consent and Eligibility Criteria

Before we do any study procedures we will ask you to sign this form. We will then check that you are eligible to take part.

Demography / Medical History

At the screening visit, the study doctor will ask you questions about your medical history. Some of this information can be obtained from your medical notes.

Respiratory Symptoms Check

At all of the visits we will ask you questions about your respiratory symptoms to see how you are going on the trial and to make sure there are no problems.

Height & weight, vital signs and physical examination

At each clinic visit the study doctor will perform a physical examination on you, including chest sounds, height and weight. We will also record your vital signs (blood pressure, heart rate, respiratory rate and temperature). The study doctor will be happy to tell you and your doctor of any findings that may need further medical attention.

Spirometry

At each clinic visit you will do a lung function test before and after a dose of your medication to see how you are progressing on the study and to make sure there are no problems caused by the medication.

Sputum collection

You will be asked to provide a sputum sample before you start the study, then at visits 2, 3, 5 and follow-up. To make sure that all the sputum samples on the study are the same, we will do sputum induction on all patients at all visits where we collect sputum. Sputum induction means that we will give you 3% hypertonic saline to breathe in order to help you to cough up the sputum in your lungs.

Blood Collection

You will have a blood test before you start the study, then at visits 2, 3, 5 and follow-up. You will be offered numbing cream so that the tests don't hurt. Again, the blood test is to make sure that everything is going well on the trial.

Concomitant Medications and Adverse Events

At every visit we will ask about the medications that you are taking and about any health events that you experience while you are on the study.

First Dose of Study Medication and Patient Observation

You will take your first dose of study medication while you are at the hospital. We do not expect you to notice any difference between the inhaled medication used in the study and the inhaled medication you normally take. It should taste and feel exactly the same. However, just to make sure that everything goes well, the research staff, including the doctor, will be there. In particular, the doctor will listen to your chest, check your breathing, and ask you to do blowing tests (spirometry) three times in the first couple of hours after your first dose. This again, is just to make sure the inhaled medication has no abnormal effect on your breathing.

Dispense Medication

During the rest of your hospital stay, the nurses will give you your study medication each day in addition to all your usual medications. Nothing else will change. When you are discharged from hospital, you will then be given your four weeks' supply of inhaled medication to take home with you. Please keep all unused study medication and used containers and bring them back at your next visit.

Will this study benefit me?

If the medication is successful, it should improve the effect of treating the *PsA* infection, and this will help reduce the damage this bacteria causes to the lung.

Will this study benefit other people?

If this study shows that tobramycin plus EDTA helps treat *PsA* lung infections better than tobramycin alone, then other people with CF may benefit in the future.

What are the possible risks?

While it is always possible for any inhaled drug to cause a reaction in your lungs, we think it is extremely unlikely with this one. Previous studies in children inhaling this drug are limited although there was one study where children inhaled EDTA twice daily for three months with no adverse effects. At the most you could experience some cough and possibly tight chest and wheeze. Ventolin should fix this very quickly. and we will explain how this should be used if it becomes necessary. Nevertheless, we have provided you with phone numbers (see below) to ring at any time if you think you are experiencing any adverse effects or allergic reactions from this medicine.

Inhaled medications are far less likely to cause systemic (generalised body) adverse effects as most (or sometimes, all) of the medication stays in the lungs and only a small amount of

Ca-EDTA is likely to be absorbed. In the past, Ca-EDTA has been given intravenously to children in much larger doses than will be used in this study, without causing any significant adverse effects.

We recommend that you report any untoward symptoms you experience on this study to a member of the study team.

Risks Related to Study Procedures

Sputum Sample Collection: Obtaining a good sputum specimen is very important in order to get accurate results. To help you produce a good sputum specimen, we will be giving you a nebuliser containing hypertonic saline before trying to collect the specimen. Breathing this hypertonic saline will probably make you cough and this will help to bring up a good sputum specimen. You may also get a dry mouth, chest tightness, nausea (feeling like you want to throw up) or excess salivation (producing a lot of spit). Inhaled Ventolin can be given to help these symptoms if they are uncomfortable.

Blood Sample Collection: When blood samples are taken from a vein, you may have discomfort or pain where the blood was taken. Sometimes a person may become dizzy or faint when blood is taken. There is also a risk of infection (rare), bleeding, redness or bruising at the skin puncture. Bleeding and bruising can usually be reduced by putting pressure on the place where the blood was taken. The chance of infection is lowered by using standard skin cleaning and sterile needles. You will be offered numbing cream so that you can't feel the blood test

Spirometry (Lung Function Tests): Since you must blow hard several times for this test, you could cough or feel short of breath during or after the test.

Other risks

The treatment and procedures involved in this research study may involve unexpected risks that are impossible to predict. These unforeseen risks may affect you while you are participating in the study or at some point in the future. If we find out any new information about the study treatment that may affect your willingness to take part in this study, we will let you know what that information is. You can then decide whether you still want to do the study.

What happens if I am injured as a result of participating in this study?

You will be compensated if you suffer an injury as a result of your participation in this research project. Compensation will be provided in accordance with the Medicines Australia (formerly known as APMA) Guidelines for compensation for injury resulting from participation in a company-sponsored clinical trial subject to the scope of the conditions "No-Fault

Compensation Insurance for Clinical Trials". A copy of the Medicines Australia Guidelines is available to you from the research staff on request.

Can I withdraw from the study?

You can withdraw from this study at any time. If you decide to withdraw from this study please inform a member of the study team. The study team may still want to use the information and samples they have already collected from you. For safety reasons, they may also ask to see you for a follow-up visit. There will be no penalty or loss of benefits in your routine medical care or any other benefit that you are entitled to receive.

Can someone else withdraw me from the study?

You may be taken off the study for various reasons. These reasons include, but are not limited to, the following:

- Dr Clements determines that it is in your best interest not to continue
- You are unable to complete required study treatments and examinations
- The study is stopped by Princess Margaret Hospital, the Sponsor, the Therapeutic Goods Administration (TGA) or other health authority in Australia
- The study is halted for safety reasons.

Will I be paid for taking part in the study?

You will not receive payment for taking part in this study. However you will be re-imbursed $75.00 for each visit where you have to travel to the hospital, in order to cover travel expenses, parking, etc.

Where is your information kept?

During the study the study doctor and study staff will collect and record information about you. This information will be transferred to a secure electronic database so that researchers can analyse it. Identifying information, such as your name or address, will not be stored in the database. You will be identified by a code that is assigned by the study staff. Information from this study will be kept by the Department of Respiratory Medicine at Princess Margaret Hospital for Children for a period of 25 years from the time when the last patient completes the study.

What about my privacy?

We hope to publish the results of this research study so that other CF clinics in Australia and New Zealand will be able to benefit. Information contained in your medical and research records will remain confidential to the extent permitted by law. Efforts will be made to keep your personal information confidential. However, we cannot guarantee complete confidentiality. You will be identified by a code, and personal information from your records

will not be released without your written permission. Results may be discussed at conferences or may be published, but you will not be identified.

The study will be conducted in accordance with recognised international quality standards, the International Conference on Harmonisation – Good Clinical Practice (ICH-GCP) guidelines. According to ICH-GCP guidelines the accuracy of information recorded for a study must be checked against source data (wherever information is recorded originally, for example your medical records, laboratory test results etc.) in order to ensure the results of the study are reliable and that study procedures were conducted correctly. By signing the informed consent form, you are giving your permission for authorised representatives of the study sponsor monitor(s), auditor(s), the ethics committee and domestic and foreign health authorities to be granted direct access to your original medical records and other source data to the extent permitted by the applicable laws and regulations.

Who has approved the study?

This study has been approved by the Princess Margaret Hospital Ethics Committee.

Will I be told about the results when the study is finished?

We will send you a letter with the results of the study when it is finished, however, it may take some time before we are able to do this.

Who can I contact for more information about this study?

If you would like any more information about this study, please do not hesitate to contact a member of the research team. They are very happy to answer your questions.

Name	Contact Number	Position
Dr Barry Clements	(08) 9340 8830	Principal Investigator
Dr Ramaa Puvvadi	(08) 9340 8830	Respiratory Physician
Anneli Robbshaw	(08) 9489 7819	Trial Coordinator
Annemarie Naylor	(08) 9489 7820	Trial Coordinator
Lucy McCahon	(08) 9489 7820	Trial Coordinator
Emergency (after hours)	(08) 9340 8222	PMH switchboard – ask for Emergency Department

Who do I contact if I have concerns about the organisation or running of the study?

If you have any concerns or complaints regarding this study, you can contact the Director of Medical Services at PMH (Telephone No: (08) 9340 8222). Your concerns will be drawn to the attention of the Ethics Committee who is monitoring the study.

Research Study - Consent Form

Study Title	A Phase IIb, Single-Centre, Randomised, Double-Blind, Comparator-Controlled, Parallel-Group, Pilot Study of Ca-EDTA added to Inhaled Tobramycin vs Tobramycin Alone as Adjunctive therapy to a Course of Standard Treatment for Cystic Fibrosis Children Admitted to Hospital with a *Pseudomonas aeruginosa* Pulmonary Exacerbation.

Declaration by participant or parent / guardian of participant

I have read the information about this study.

I understand the purpose, procedures and risks of participating in this study.

I have had an opportunity to ask questions about this study and I am satisfied with the answers I have received.

I agree to participate in this study and understand that I am free to withdraw at any time without affecting my current or future health care.

I understand that I will be given a signed copy of this document to keep.

Name:

_____

Signature: **Date:**

_____ _____

Child Assent

I would like to take part in this study:

☐ Yes

☐ No

OR

Not Applicable:

☐ Participant is an adult

☐ Child is not yet able to give assent

Name:

_____

Signature: _____ **Date:** _____

Declaration by person obtaining consent

I explained this study, its procedures and risks and I believe that the participant or parent / guardian of the participant has understood that explanation.

Name:

_____

Signature: _____ **Date:** _____

Research Study

Withdrawal of Consent

Study Title	A Phase IIb, Single-Centre, Randomised, Double-Blind, Comparator-Controlled, Parallel-Group, Pilot Study of Ca-EDTA added to Inhaled Tobramycin vs Tobramycin Alone as Adjunctive therapy to a Course of Standard Treatment for Cystic Fibrosis Children Admitted to Hospital with a *Pseudomonas aeruginosa* Pulmonary Exacerbation.

Declaration by participant or parent / guardian of participant

I wish to withdraw from participation in the above study.

I understand that my withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with Princess Margaret Hospital.

I understand that the information and samples already collected on the study may still be used and shared as described in the study information sheet.

Name:

_____

Signature: **Date:**

_____ _____

Declaration by person who received the withdrawal of consent

I have explained the implications of withdrawing from the study and I believe that the participant or parent / guardian of the participant has understood my explanation.

Name:

_____

Signature: **Date:**

_____ _____

12.2 Child information sheet

Government of **Western Australia**
Department of **Health**
Child and Adolescent Health Service

Research Study

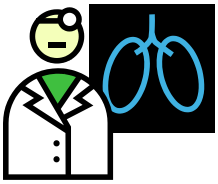
Child Information Sheet

Study Title	A Phase IIb, Single-Centre, Randomised, Double-Blind, Comparator-Controlled, Parallel-Group, Pilot Study of Inhaled Tobramycin with added Ca-EDTA vs Tobramycin Alone as Adjunctive therapy to a Course of Standard Treatment for Cystic Fibrosis Children Admitted to Hospital with a *Pseudomonas aeruginosa* Pulmonary Exacerbation.
Study ID	TEDIV-001
Protocol Version	Protocol version 2.0 dated 16 August 2013
Principal Investigator	Dr Barry Clements
Institution	Princess Margaret Hospital for Children
Phone Number	9340 8830 or 9340 8222 (24-hr emergency)

Why are we doing the study?

We're asking if you would like to do this study because you have cystic fibrosis (CF) and it looks like you have a germ called *Pseudomonas* in your lungs. We would like to try a new way of treating this type of germ by adding an extra chemical called EDTA to your nebuliser medicine.

Who is running the study?



Dr Clements and other people who work at Princess Margaret Hospital (PMH) are in charge of doing the study. We will ask your normal doctor if he/she is happy for you to participate in the study. He/she will still be looking after you.

What will the study tell us?

We think that adding EDTA to your nebuliser medicine will stop the germs in your lungs from making a slime they use to protect themselves. If we get rid of the slime then it will be easier to kill the germs.

Do I have to do this study?

You don't have to do this study if you don't want to. If you start the study, you can ask to stop at any time. Whatever you decide, you will be properly looked after.

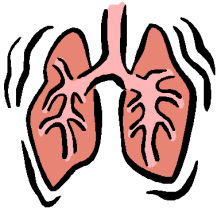
What will I have to do if I go on this study?

We will ask you lots of questions while you are on this study and we will also do some tests. The tests we will ask you to do are:

- Measure your height and weight.
- Measure your blood pressure. We do this by putting a cuff on your arm. A machine will then make the cuff puff up with air. All the air then leaves the cuff. This allows the machine to measure your blood pressure.
- Measure how fast your heart is beating. This is done at the same time the machine measures your blood pressure.
- Measure your temperature. We do this with a thermometer that goes in your ear. It takes about a second and will not hurt.
- Measure how fast you are breathing and listen to your chest.
- Do a lung function test. This is the same test you normally do in clinic.
- Cough up some sputum. We will ask you to do this at most of the visits. We will give you some hypertonic saline (salty water) to breathe through a nebuliser to help you cough up the sputum.
- Blood tests. These also happen at most visits (five times altogether). We can put some numbing cream on your arm so that you don't feel the blood test.

You will also have to take the study medication every day for the whole study. This will be exactly like taking your normal nebuliser medicine.

Will doing this study help me?



We do not know if doing this study will help you. The study medicine may be better at killing the germs in your lungs than your normal medicine.

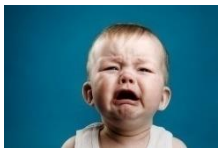
Will doing this study help other people?

If we find out that the study medicine is better at killing the germs in your lungs, this might help us get better treatments for other people with cystic fibrosis.

Can anything bad happen to me on this study?

We don't think that anything bad will happen to you on this study. Adding the chemical EDTA to your normal nebuliser medicine shouldn't make it any different.

Will anything hurt or be uncomfortable if I do this study?

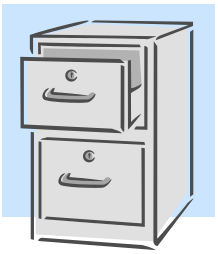


Breathing hypertonic saline when we are collecting sputum will probably make you cough. You may also get a dry mouth, your chest might feel tight, you might feel a little sick (like you want to throw up) or you might start making a lot of spit.

Doing the breathing test might make you cough or feel a little funny.

When you have blood taken with a needle, it may feel like a pinch. Sometimes the place where the needle goes might get red and sore. Having a blood test also makes some people feel a bit funny. If any of this happens, tell the doctor or your parent or guardian right away.

Where will my information be kept?



Your private information will be stored in a locked filing cabinet or on a computer that is password protected.

What about my privacy?

We will keep your information as private as possible. Instead of writing your name on the study papers, we will give you a code instead. Only the people who run the study will know that the code is yours.

Who has approved the study?

This study has been approved by the Princess Margaret Hospital Ethics Committee.

Who should I contact for more information about this study?

If you want more information about this study, please contact one of the people on the research team. They are happy to answer your questions.

Name	Title	Contact Number
Dr Barry Clements	Respiratory Doctor	(08) 9340 8830
Dr Ramaa Puvvadi	Respiratory Doctor	(08) 9340 8830
Anneli Robbshaw	Trial Coordinator	(08) 9489 7819
Annemarie Naylor	Trial Coordinator	(08) 9489 7820
Lucy McCahon	Trial Coordinator	(08) 9489 7820

Who should I contact if I am worried about the way the study was run?

If you are worried or want to complain about this study, you can contact the Director of Medical Services at PMH on (08) 9340 8222. They will then tell the PMH Ethics Committee (the people who approved this study).

What should I do if I want to take part in this research?

Tell your parents so that they can sign the forms that will allow you take part.

THANK YOU FOR YOUR TIME! ☺

12.3 Sample CFQ-R questionnaire

TRIAL ID: TED IV

PATIENT INITIALS: ___ ___ ___

SCREENING

PATIENT ID: TED IV - ___ ___ ___

QUESTIONNAIRE						
Since your last visit:		**0**	**1**	**2**	**3**	**4**
Activity	Have there been any changes in your ability to do physical activity?	Much easier ☐	A little easier ☐	No change ☐	A little harder ☐	Much harder ☐
Cough	Have there been any changes in how heavily you've been coughing?	Much lighter ☐	A little lighter ☐	No change ☐	A little heavier ☐	Much heavier ☐
	Have there been any changes in how often you've been coughing?	Much less often ☐	A little less often ☐	No change ☐	A little more often ☐	Much more often ☐
Sputum	Have there been any changes in the amount of sputum you have been coughing up?	Much less ☐	A little less ☐	No change ☐	A little more ☐	Much more ☐
	Have there been any changes in the colour of your sputum?	Clear ☐	Clear to yellow ☐	Yellowish-green ☐	Green ☐	Brown ☐
TOTAL						

VITAL SIGNS	
Height: __ __ __ . __ cm	Weight: __ __ . __ kg
Heart rate: __ __ __ beats/min	Respiratory rate: __ __ breaths/min
Temperature (tympanic): __ __ . __ °C	O_2 saturation: __ __ __ %

TRIAL ID: TED IV PATIENT INITIALS: ___ ___ ___

SCREENING PATIENT ID: TED IV - ___ ___ ___

PHYSICAL EXAMINATION				
Assessment	**Normal**	**Abnormal**	**Not done**	**Findings**
Skin	☐	☐	☐	
Head, eyes, ears, nose and throat	☐	☐	☐	
Respiratory	☐	☐	☐	
Cardiovascular	☐	☐	☐	
Gastrointestinal	☐	☐	☐	
Neurological	☐	☐	☐	
Musculoskeletal	☐	☐	☐	
Other, specify: _____	☐	☐	☐	
PE performed by: _____				Date: __ __ / __ __ __ / __ __ __ __

Signed: _____	Date: __ __ / __ __ __ / __ __ __ __

TRIAL ID: TED IV

PATIENT INITIALS: ___ ___ ___

SCREENING

PATIENT ID: TED IV - ___ ___ ___

NOTES

TRIAL ID: TED IV

PATIENT INITIALS: ___ ___ ___

SCREENING

PATIENT ID: TED IV - ___ ___ ___

Signed: _____	Date: __ __ / __ __ __ / __ __ __ __

12.4 Patient data listings

12.4.1 Protocol deviations

Patient ID	Deviation
TEDIV-001-07	Mother consented with the patient's assent, but patient did not want to cooperate during the screening visit and was withdrawn by study team before any study drug was administered.
TEDIV-001-08	After 7 days on the study drug, the patient discharged herself and the study team was unable to make contact again. Patient was withdrawn by the study team due to non-compliance.
TEDIV-001-09	Mother consented with patient's assent, but patient changed her mind and mother decided to withdraw on day 2.
TEDIV-001-11	Patient experienced a pulmonary exacerbation after 4 weeks of treatment and stopped taking the study drug four days early to commence treatment with another antibiotic. Because of this event, visit 5 was conducted 4 days outside the visit window.
TEDIV-001-12	Patient did not attend visit 2 due to a hernia repair. As much information as possible was collected by telephone.
TEDIV-001-12	Patient presented with an episode of hemoptysis and study drug was interrupted for 12 hours on day 13.
TEDIV-001-16	Patient did not attend visit 2 at PMH because he was admitted at SCGH, but continued the study.
TEDIV-001-17	Patient did not attend visit 2 at PMH because he was admitted at SCGH, but continued the study.
TEDIV-001-19	Patient requested more time to consider whether to participate in the study and started the study drug 17 hours after the window set by the protocol.
TEDIV-001-21	Patient went abroad on holiday and, as a result, was not available for visit 6, which was conducted 15 days outside the study window.
TEDIV-001-23	Patient did not attend visit 2 because he was receiving outpatient treatment 230 km away from the study site. As much information as possible was collected by telephone.
TEDIV-001-23	Due to the Christmas holiday season, patient was unable to attend visit 6 as planned, and the visit was conducted one day outside the study window.
TEDIV-001-24	Patient did not attend visit 2 because she was receiving care at home on the Hosptal in the Home-program, but continued on the study.
TEDIV-001-25	Visit 4 telephone call was not conducted, because the study team was unable to get hold of the patient's mother.
TEDIV-001-25	Due to the Christmas holiday season, patient was unable to attend the end-of-treatment visit as planned, and visit 5 was conducted 5 days outside the study window.

12.4.2 Demographic data

Subject_ID	Group	Age	Gender	Ethnicity	Admissions in previous 12 months	Pancreatic status	Site
TEDIV-001-01	CaEDTA	12	F	Aboriginal	2	Insufficient	PMH
TEDIV-001-02	Saline	15	F	Aboriginal	2	Insufficient	PMH
TEDIV-001-03	Saline	12	M	Caucasian	3	Insufficient	PMH
TEDIV-001-04	CaEDTA	16	M	Caucasian	0	Insufficient	PMH
TEDIV-001-05	Saline	10	F	Caucasian	2	Insufficient	PMH
TEDIV-001-06	CaEDTA	17	M	Caucasian	0	Insufficient	PMH
TEDIV-001-08	Saline	14	F	Aboriginal	1	Insufficient	PMH
TEDIV-001-09	CaEDTA	15	F	Caucasian	2	Insufficient	PMH
TEDIV-001-10	Saline	16	F	Caucasian	1	Insufficient	PMH
TEDIV-001-11	Saline	16	F	Caucasian	1	Insufficient	PMH
TEDIV-001-12	CaEDTA	24	M	Caucasian	0	Insufficient	SCGH
TEDIV-001-13	CaEDTA	9	F	Caucasian	0	Insufficient	PMH
TEDIV-001-15	Saline	7	M	Caucasian	1	Insufficient	PMH
TEDIV-001-16	CaEDTA	38	M	Caucasian	1	Insufficient	SCGH
TEDIV-001-17	Saline	30	M	Caucasian	0	Sufficient	SCGH
TEDIV-001-18	CaEDTA	7	M	Caucasian	2	Insufficient	PMH
TEDIV-001-19	Saline	32	M	Caucasian	1	Insufficient	SCGH
TEDIV-001-20	CaEDTA	35	M	Caucasian	3	Insufficient	SCGH
TEDIV-001-21	CaEDTA	33	M	Caucasian	2	Insufficient	SCGH
TEDIV-001-22	Saline	35	M	Caucasian	0	Sufficient	SCGH
TEDIV-001-23	Saline	28	M	Caucasian	0	Insufficient	SCGH
TEDIV-001-24	CaEDTA	15	F	Caucasian	3	Insufficient	PMH
TEDIV-001-25	Saline	12	F	Caucasian	0	Insufficient	PMH
TEDIV-001-26	CaEDTA	46	M	Caucasian	0	Insufficient	SCGH

12.4.3 Compliance data

Subject ID	No. doses 0-2 weeks	% compliance 0-2 weeks	No. doses 2-6 weeks	% compliance 2-6wks
TEDIV-001-01	47	84	0	0
TEDIV-001-02	55	98	56	100
TEDIV-001-03	46	82	52	93
TEDIV-001-04	53	95	54	96
TEDIV-001-05			48	86
TEDIV-001-06	56	100	54	96
TEDIV-001-08				
TEDIV-001-09				
TEDIV-001-10	52	93	55	98
TEDIV-001-11	56	100	43	77
TEDIV-001-12	42	75	49	88
TEDIV-001-13	56	100	56	100
TEDIV-001-15	49	88	56	100
TEDIV-001-16	56	100		
TEDIV-001-17	53	95	49	88
TEDIV-001-18	55	98		
TEDIV-001-19	54	96		
TEDIV-001-20	50	89		
TEDIV-001-21	51	91	50	89
TEDIV-001-22	54	96	51	91
TEDIV-001-23			56	100
TEDIV-001-24	47	84	52	93
TEDIV-001-25	55	98	47	84
TEDIV-001-26	47	84		

12.4.4 Efficacy data – colony forming units per gram sputum

Patient ID	Cohort	Screening	Visit 3	Visit 5	Visit 6	Comment
TEDIV-001-01	CaEDTA	1.01E+05	NS	2.09E+05*	2.93E+06*	Excluded after 2 weeks due to non-compliance
TEDIV-001-02	Placebo	1.69E+05	1.42E+06	3.68E+03	NS	
TEDIV-001-03	Placebo	1.81E+04	0.00E+00	NS	NS	
TEDIV-001-04	CaEDTA	9.13E+05	8.97E+02	NS	9.17E+04	
TEDIV-001-05	Placebo	1.45E+02	0.00E+00	0.00E+00	NS	
TEDIV-001-06	CaEDTA	0.00E+00	0.00E+00	NS	NS	No culture throughout
TEDIV-001-07	CaEDTA	NS	NS	NS	NS	Dropout after Screening
TEDIV-001-08	Placebo	1.02E+06*	NS	NS	NS	Withdrawn on day 8
TEDIV-001-09	CaEDTA	2.55E+05*	NS	NS	NS	Withdrawn on day 2
TEDIV-001-10	Placebo	8.57E+06	1.24E+08	7.92E+05	9.44E+06	
TEDIV-001-11	Placebo	9.59E+05	3.99E+05	1.58E+05	1.86E+05	
TEDIV-001-12	CaEDTA	5.57E+04	0.00E+00	1.43E+03	2.56E+04	
TEDIV-001-13	CaEDTA	1.54E+03	NS	0.00E+00	NS	
TEDIV-001-14	Placebo	NS	NS	NS	NS	Dropout after screening
TEDIV-001-15	Placebo	0.00E+00	2.50E+02	NS	NS	
TEDIV-001-16	CaEDTA	4.46E+05	1.16E+05	4.17E+04	1.02E+04	
TEDIV-001-17	Placebo	1.05E+05	0.00E+00	0.00E+00	2.52E+04	
TEDIV-001-18	CaEDTA	1.79E+06	NS	NS	NS	
TEDIV-001-19	Placebo	2.42E+04	1.36E+05	NS	0.00E+00	
TEDIV-001-20	CaEDTA	5.15E+05	3.96E+04	NS	NS	
TEDIV-001-21	CaEDTA	0.00E+00	0.00E+00	2.24E+04	NS	
TEDIV-001-22	Placebo	0.00E+00	NS	2.25E+02	NS	
TEDIV-001-23	Placebo	NS	0.00E+00	0.00E+00	0.00E+00	No culture throughout
TEDIV-001-24	CaEDTA	1.42E+07	1.41E+08	1.27E+05	NS	
TEDIV-001-25	Placebo	NS	NS	NS	NS	No sample throughout
TEDIV-001-26	CaEDTA	7.99E+05	0.00E+00	2.83E+03	3.63E+06	

*Data not used for analysis as explained in "Comment"

12.4.5 Efficacy and safety data – FEV1

Subject_ID	Group	Visit 1 Pre-dose	Visit 1 30 min*	Visit 1 1 hour*	Visit 1 2 hours*	Visit 3	Visit 5	Visit 6
TEDIV-001-01	CaEDTA	23	25		32	75	56	57
TEDIV-001-02	Saline	79	73	75	76	103	99	85
TEDIV-001-03	Saline	82	71	76	87	79	107	105
TEDIV-001-04	CaEDTA	72	67		73	67	73	75
TEDIV-001-05	Saline	62		56	62	48	40	54
TEDIV-001-06	CaEDTA	97	91	95	98	99	97	99
TEDIV-001-08	Saline	55	45					
TEDIV-001-09	CaEDTA	78	78	77	80			
TEDIV-001-10	Saline	87	87	88	87	83	79	87
TEDIV-001-11	Saline	79		76	80	90	81	85
TEDIV-001-12	CaEDTA	86	86	88	86	86	75	76
TEDIV-001-13	CaEDTA	85	91	84	87	112	107	97
TEDIV-001-15	Saline	100				116	95	99
TEDIV-001-16	CaEDTA	37	35	33		38	41	37
TEDIV-001-17	Saline	76			75	77	77	77
TEDIV-001-18	CaEDTA	55				93	97	
TEDIV-001-19	Saline	30		32		38		33
TEDIV-001-20	CaEDTA	23	22	20	19	24	19	18
TEDIV-001-21	CaEDTA	40		32	32	42	43	56
TEDIV-001-22	Saline	58	57	55	57	60	58	49
TEDIV-001-23	Saline	41	40	38	42	53	51	48
TEDIV-001-24	CaEDTA	32				83	81	68
TEDIV-001-25	Saline	103	103	97	94	104	99	95
TEDIV-001-26	CaEDTA	32	32	32		35	32	29

*Post-dose measurements during visit 1

12.4.6 Efficacy data – CFQ-R questionnaire

CFQ-R		Visit			
Subject_ID	**Group**	**Screening**	**Visit 3**	**Visit 5**	**Visit 6**
TEDIV-001-01	CaEDTA	18		11	15
TEDIV-001-04	CaEDTA	15	9	14	16
TEDIV-001-06	CaEDTA	13	13	13	13
TEDIV-001-09	CaEDTA	19			
TEDIV-001-12	CaEDTA	21	22	19	18
TEDIV-001-13	CaEDTA	17	15	11	13
TEDIV-001-16	CaEDTA	19	14	17	18
TEDIV-001-18	CaEDTA	22	16	22	13
TEDIV-001-20	CaEDTA	17	9	17	11
TEDIV-001-21	CaEDTA	18	8	20	12
TEDIV-001-24	CaEDTA	20	7	14	10
TEDIV-001-26	CaEDTA	22	20	24	23
TEDIV-001-02	Saline	21	13	21	
TEDIV-001-03	Saline	18	11	5	7
TEDIV-001-05	Saline	19	20	18	17
TEDIV-001-08	Saline	20			
TEDIV-001-10	Saline	19	12	12	11
TEDIV-001-11	Saline	19	6	23	20
TEDIV-001-15	Saline	17	11	5	12
TEDIV-001-17	Saline	14	17	16	14
TEDIV-001-19	Saline	25	8	15	15
TEDIV-001-22	Saline	19	8	11	19
TEDIV-001-23	Saline	20	8	17	16
TEDIV-001-25	Saline	14	15	15	13

12.4.7 Adverse events

Subject ID	Treatment	Hemoptysis	Headache	Nausea	Vomiting	Cough	Sore Throat	Abdomen Pain	Rash	Other
TEDIV-001-01	CaEDTA		one day	one day			10 days	one day		
TEDIV-001-02	Placebo		mild for 7 days			mild increase				
TEDIV-001-03	Placebo		intermittent					creon mismatch-related	rash on hands and oral thrush	
TEDIV-001-04	CaEDTA									
TEDIV-001-05	Placebo				two episodes		one day		rash with viral infection	admission to hospital, viral infection with abnormal LFT's
TEDIV-001-06	CaEDTA		mild intermittent							
TEDIV-001-08	Placebo									
TEDIV-001-09	CaEDTA									
TEDIV-001-10	Placebo	mild increase[1]	mild intermittent							
TEDIV-001-11	Placebo		headache			increased cough, stopped treatment early[2]				epistaxis
TEDIV-001-12	CaEDTA	increased frequency[3]								
TEDIV-001-13	CaEDTA		mild for 1 hour					abdomen pain for 1 hour		
TEDIV-001-15	Placebo								rash from ceftazidime allergy	
TEDIV-001-16	CaEDTA									flu-like symptoms for 1 week
TEDIV-001-17	Placebo				vomiting with viral URTI					
TEDIV-001-18	CaEDTA									

Subject ID	Treatment	Hemoptysis	Headache	Nausea	Vomiting	Cough	Sore Throat	Abdomen Pain	Rash	Other
TEDIV-001-19	Placebo									admitted during follow-up period with a viral URTI
TEDIV-001-20	CaEDTA		mild, usual for patient							
TEDIV-001-21	CaEDTA									
TEDIV-001-22	Placebo	occasional					yes			flu-like symptoms
TEDIV-001-23	Placebo					increased				
TEDIV-001-24	CaEDTA									icterus, likely from tazocin
TEDIV-001-25	Placebo						yes	yes		cervical lymphadenopathy
TEDIV-001-26	CaEDTA									chest tightness related to risedronate, dizzy after coughing

[1]Mild increase in hemoptysis experienced during the study period; not specifically related to treatment, since it was a common feature for this participant and remained mild (<5ml).

[2]Increase in moist cough due to viral illness; patient requested to stop treatment four days early due to tiredness, but remained a participant and completed the study.

[3]Increased frequency of hemoptysis, moderate on two occasions.

12.4.8 Laboratory data – blood tests

Creatinine		**Visit**			
Subject ID	**Cohort**	**Screening**	**Visit 3**	**Visit 5**	**Visit 6**
TEDIV-001-01	CaEDTA	39			
TEDIV-001-02	Saline	46	47	52	50
TEDIV-001-03	Saline	44	47	39	38
TEDIV-001-04	CaEDTA	42	51	54	57
TEDIV-001-05	Saline	32	27	35	40
TEDIV-001-06	CaEDTA	47	48		52
TEDIV-001-08	Saline	55			
TEDIV-001-09	CaEDTA	50			
TEDIV-001-10	Saline	34	41	42	40
TEDIV-001-11	Saline	44	51	42	42
TEDIV-001-12	CaEDTA	77	100	68	73
TEDIV-001-13	CaEDTA	34	36	29	52
TEDIV-001-15	Saline	25	24	77	43
TEDIV-001-16	CaEDTA	68			
TEDIV-001-17	Saline	78	87		88
TEDIV-001-18	CaEDTA	28	27	32	
TEDIV-001-19	Saline	84	79	76	73
TEDIV-001-20	CaEDTA	78	72	65	58
TEDIV-001-21	CaEDTA	69	81	71	76
TEDIV-001-22	Saline	88	92	88	88
TEDIV-001-23	Saline	85	93	70	79
TEDIV-001-24	CaEDTA	73	67	59	55
TEDIV-001-25	Saline	58	46	58	
TEDIV-001-26	CaEDTA	77	70	82	

Alanine aminotransferase		Visit			
Subject ID	**Cohort**	**Screening**	**Visit 3**	**Visit 5**	**Visit 6**
TEDIV-001-01	CaEDTA	37			
TEDIV-001-02	Saline	20	46	25	24
TEDIV-001-03	Saline	28	49	45	38
TEDIV-001-04	CaEDTA	39	53	68	35
TEDIV-001-05	Saline	42	79	155	66
TEDIV-001-06	CaEDTA	59	95	52	52
TEDIV-001-08	Saline	11			
TEDIV-001-09	CaEDTA	14			
TEDIV-001-10	Saline	40	49	40	33
TEDIV-001-11	Saline	24	26	34	18
TEDIV-001-12	CaEDTA	41	32	39	41
TEDIV-001-13	CaEDTA	20	25	28	25
TEDIV-001-15	Saline	153	78	55	46
TEDIV-001-16	CaEDTA	72			
TEDIV-001-17	Saline	29		77	41
TEDIV-001-18	CaEDTA	46	26	26	
TEDIV-001-19	Saline	21	71	17	56
TEDIV-001-20	CaEDTA	16	34	20	14
TEDIV-001-21	CaEDTA	19	46	12	12
TEDIV-001-22	Saline	21	21	11	37
TEDIV-001-23	Saline	38	107	68	
TEDIV-001-24	CaEDTA	18	9	23	24
TEDIV-001-25	Saline	7	24	74	
TEDIV-001-26	CaEDTA	29	39	29	

Gamma-Glutamyl Transferase		Visit			
Subject ID	**Group**	**Screening**	**Visit 3**	**Visit 5**	**Visit 6**
TEDIV-001-01	CaEDTA	22			
TEDIV-001-02	Saline	12	17	22	18
TEDIV-001-03	Saline	21	37	27	18
TEDIV-001-04	CaEDTA	52	64	63	49
TEDIV-001-05	Saline	30	66	150	31
TEDIV-001-06	CaEDTA	36	51	37	37
TEDIV-001-08	Saline	22			
TEDIV-001-09	CaEDTA	18			
TEDIV-001-10	Saline	56	57	55	52
TEDIV-001-11	Saline	18	23	27	24
TEDIV-001-12	CaEDTA	40	33	41	41
TEDIV-001-13	CaEDTA	10	16	21	10
TEDIV-001-15	Saline	112	118	126	96
TEDIV-001-16	CaEDTA	197			
TEDIV-001-17	Saline	17			
TEDIV-001-18	CaEDTA	25	30	30	
TEDIV-001-19	Saline	23	34	23	
TEDIV-001-20	CaEDTA	102	102	144	114
TEDIV-001-21	CaEDTA	25	21	33	33
TEDIV-001-22	Saline	15	25	20	23
TEDIV-001-23	Saline	44	36	46	
TEDIV-001-24	CaEDTA	25	29	15	16
TEDIV-001-25	Saline	11	11	22	
TEDIV-001-26	CaEDTA	95	103	213	

Iron Studies	Analysis	Ferritin				Iron				Transferrin				Transferrin sat.			
Subject ID	**Group**	**SCR**	**V3**	**V5**	**V6**	**SCR**	**V3**	**V5**	**V6**	**SCR**	**V3**	**V5**	**V6**	**SCR**	**V3**	**V5**	**V6**
TEDIV-001-01	CaEDTA	24				15				31				24			
TEDIV-001-02	Saline	8	110	18	13	6	32	10	12	32	34	34	32	9	47	19	19
TEDIV-001-03	Saline	46	26	15	12	14	20	20	20	30	37	39	37	23	27	26	27
TEDIV-001-04	CaEDTA	42	18	28	25	7	27	11	13	27	35	33	30	13	39	17	22
TEDIV-001-05	Saline	13	17		15	12	10		16	40	41		41	15	12		20
TEDIV-001-06	CaEDTA	44	44	58	58	24	28	21	21	26	27	27	27	46	52	39	39
TEDIV-001-08	Saline	5				6				32				9			
TEDIV-001-09	CaEDTA					13				32							
TEDIV-001-10	Saline	54	23	44	29	6	8	9	8	33	36	39	36	9	11	12	11
TEDIV-001-11	Saline	19	11	25	24	8	25	8	6	29	32	35	30	14	39	11	10
TEDIV-001-12	CaEDTA	87	158	120	82	18	10	16	12	36	33	32	30	25	15	25	20
TEDIV-001-13	CaEDTA	25	29	26	23	5	21	15	9	29	30	31	35	9	35	24	13
TEDIV-001-15	Saline	17	23	44	19	15	27	14	23	33	35	38	37	23	39	18	31
TEDIV-001-16	CaEDTA	37		44	63	7				33				11		44	
TEDIV-001-17	Saline	233				10				20				25			
TEDIV-001-18	CaEDTA	18	23	26	26	11	20	20		32	37	37		17	27	27	
TEDIV-001-19	Saline	138	83	54	121	3	20	18	18	27	43	36	37	6	30	25	24
TEDIV-001-21	CaEDTA	53	99	55	34	12	11	20	29	30	30	28	32	20	18	36	45
TEDIV-001-22	Saline	194	172	166	263	14	19	21	21	26	20	23	50	27	48	46	53
TEDIV-001-23	Saline	76	70	54	49	23	15	11	10	32	36	33	34	36	21	17	15
TEDIV-001-24	CaEDTA	91	201	12	14	5	14	10	9	25	30	33	33	10	23	15	14
TEDIV-001-25	Saline	27	24			8	16			32	32			13	25		
TEDIV-001-26	CaEDTA	36		45		12		5		36		39		17		6	

Hemoglobin		Visit			
Subject ID	**Group**	**Screening**	**Visit 3**	**Visit 5**	**Visit 6**
TEDIV-001-01	CaEDTA	127			
TEDIV-001-02	Saline	107	98	127	123
TEDIV-001-03	Saline	128	133	134	132
TEDIV-001-04	CaEDTA	145	152	156	156
TEDIV-001-05	Saline	144	136	121	131
TEDIV-001-06	CaEDTA	144	147	148	155
TEDIV-001-08	Saline	132			
TEDIV-001-09	CaEDTA	138			
TEDIV-001-10	Saline	110	111	119	116
TEDIV-001-11	Saline	127	133	131	133
TEDIV-001-12	CaEDTA	149	160	148	145
TEDIV-001-13	CaEDTA	125	118		123
TEDIV-001-15	Saline	118	113	138	117
TEDIV-001-16	CaEDTA	135		138	137
TEDIV-001-17	Saline	130		138	138
TEDIV-001-18	CaEDTA	127	120	129	129
TEDIV-001-19	Saline	149	162	163	156
TEDIV-001-20	CaEDTA	117	126	136	142
TEDIV-001-21	CaEDTA	156	165	150	158
TEDIV-001-22	Saline	158	156	154	155
TEDIV-001-23	Saline	143	143	137	134
TEDIV-001-24	CaEDTA	134	100	126	135
TEDIV-001-25	Saline	123	123	134	
TEDIV-001-26	CaEDTA	135	128	135	

Blood Urea Nitrogen		Visit			
Subject_ID	**Group**	**Screening**	**Visit 3**	**Visit 5**	**Visit 6**
TEDIV-001-01	CaEDTA	3.9			
TEDIV-001-02	Saline	2.4	28	3.2	2.2
TEDIV-001-03	Saline	2.3	4.7	4.3	4.7
TEDIV-001-04	CaEDTA	3.1	5.2	3.1	3.2
TEDIV-001-05	Saline	3.4	7.2	3.1	5.3
TEDIV-001-06	CaEDTA	4	6.1		4.5
TEDIV-001-08	Saline				
TEDIV-001-09	CaEDTA				
TEDIV-001-10	Saline	2.8	2.4	3.3	2.9
TEDIV-001-11	Saline	3.7	5.9	3.9	3.9
TEDIV-001-12	CaEDTA	3.8	5.1	4.1	5.1
TEDIV-001-13	CaEDTA	3.5	6.4	4.2	
TEDIV-001-15	Saline	3.9	4.3	5.7	5.7
TEDIV-001-16	CaEDTA	4.9			
TEDIV-001-17	Saline	3.5	5.4		5.4
TEDIV-001-18	CaEDTA	0.9	4.7		
TEDIV-001-19	Saline	4.4	8	5.5	5.7
TEDIV-001-20	CaEDTA	3.2	6.9	6.3	6.8
TEDIV-001-21	CaEDTA	5.8	4.4	4.9	5.3
TEDIV-001-22	Saline	7.1	7.3	7.6	7.1
TEDIV-001-23	Saline	7.9	7.7	8.5	9.5
TEDIV-001-24	CaEDTA	2.4	5.5	2.7	3
TEDIV-001-25	Saline	3.7	3.3	3.4	
TEDIV-001-26	CaEDTA	3.9	4.3	2.8	

Calcium		Visit			
Subject_ID	**Group**	**Screening**	**Visit 3**	**Visit 5**	**Visit 6**
TEDIV-001-01	CaEDTA	2.22			
TEDIV-001-02	Saline	2.04	2.06	2.32	2.2
TEDIV-001-03	Saline	2.31	2.35	2.45	2.3
TEDIV-001-04	CaEDTA	2.23	2.27	2.3	2.23
TEDIV-001-05	Saline	2.51	2.27		2.27
TEDIV-001-06	CaEDTA	2.26	2.32	2.32	2.46
TEDIV-001-08	Saline	2.27			
TEDIV-001-09	CaEDTA	2.32			
TEDIV-001-10	Saline		2.31	2.31	2.38
TEDIV-001-11	Saline	2.17	2.38	2.3	2.36
TEDIV-001-12	CaEDTA	2.44	2.56	2.4	2.32
TEDIV-001-13	CaEDTA	2.44	2.38	2.53	2.47
TEDIV-001-15	Saline	2.38	2.32	2.19	2.37
TEDIV-001-16	CaEDTA	2.39			
TEDIV-001-17	Saline	2.3			
TEDIV-001-18	CaEDTA	2.32	2.3	2.41	
TEDIV-001-19	Saline	2.34	2.31	2.26	2.35
TEDIV-001-20	CaEDTA	2.36	2.46	2.13	2.26
TEDIV-001-21	CaEDTA	2.36	2.36	2.2	2.18
TEDIV-001-22	Saline	2.31	2.2	2.15	2.13
TEDIV-001-23	Saline	2.23	2.27	2.3	2.22
TEDIV-001-24	CaEDTA	2.06	2.26	2.17	2.28
TEDIV-001-25	Saline	2.32	2.34	2.35	
TEDIV-001-26	CaEDTA	2.03	2.21	2.39	

Magnesium		Visit			
Subject_ID	**Group**	**Screening**	**Visit 3**	**Visit 5**	**Visit 6**
TEDIV-001-01	CaEDTA	0.77			
TEDIV-001-02	Saline	0.73	0.68	0.75	0.74
TEDIV-001-03	Saline	0.78	0.73	0.75	0.77
TEDIV-001-04	CaEDTA	0.75	0.71	0.8	0.8
TEDIV-001-05	Saline	0.75	0.84		0.74
TEDIV-001-06	CaEDTA	0.73	0.72	0.72	0.81
TEDIV-001-08	Saline	0.63			
TEDIV-001-09	CaEDTA	0.72			
TEDIV-001-10	Saline		0.62	0.62	0.68
TEDIV-001-11	Saline	0.84	0.84	0.87	0.86
TEDIV-001-12	CaEDTA	0.87	1	0.82	0.8
TEDIV-001-13	CaEDTA	0.75	0.67	0.72	0.8
TEDIV-001-15	Saline	0.71	0.76	0.76	0.7
TEDIV-001-16	CaEDTA	0.82			
TEDIV-001-17	Saline	0.86			
TEDIV-001-18	CaEDTA	0.84	0.8	0.85	
TEDIV-001-19	Saline	0.73	0.89	0.78	0.99
TEDIV-001-20	CaEDTA	0.62	0.69	0.67	0.37
TEDIV-001-21	CaEDTA	0.84	0.96	0.77	0.73
TEDIV-001-22	Saline	0.85	0.77	0.78	0.83
TEDIV-001-23	Saline	0.84	0.9	0.76	0.8
TEDIV-001-24	CaEDTA	0.7	0.74	0.7	0.71
TEDIV-001-25	Saline	0.68	0.68	0.76	
TEDIV-001-26	CaEDTA	0.8		0.85	